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(71) Applicant: PROTEIN DESIGN LABS, INC. [US/US]; 3181 Port Drive, Palo Alto, CA 94304 (US).

(72) Inventor: QUEEN, Cary, L. ; 886 Roble Avenue, Menlo Park, CA 94025 (US).

(74) Agent: HANN, James, F.; Townsend and Townsend, One Market Plaza, 2000 Steuart Tower, San Francisco, CA 94105 (US).

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(54) Title: IL-2 RECEPTOR-SPECIFIC CHIMERIC ANTIBODIES

(57) Abstract

Chimeric antibodies specifically reactive with human IL-2 receptors are prepared employing recombinant DNA technology for use in, e.g., treatment of T-cell mediated disorders.

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IL-2 RECEPTOR-SPECIFIC CHIMERIC ANTIBODIES

Field of the Invention

The present invention relates generally to the combination of recombinant DNA and monoclonal antibody technologies for developing novel therapeutic and other agents and, more particularly, to the production of chimeric antibodies specific for the human interleukin-2 receptor and the use of such chimeric antibodies in treating T-cell mediated human disorders.

Background of the Invention

In mammals, the immune response is mediated by two types of cells that interact specifically with foreign material, i.e., antigens. One of these cell types, B-cells, are responsible for the production of antibodies. The second cell class, T-cells, include a wide variety of cellular subsets controlling the in vivo function of both B-cells and a wide variety of other hematopoietic cells, including T-cells.

One way in which T-cells exert this control is through the production of a lymphokine known as interleukin-2 (IL-2), originally named T-cell growth factor. IL-2's prime function appears to be the stimulation and maintenance of T-cells. Indeed, some immunologists believe that IL-2 may be at the center of the entire immune response (see, Farrar, J., et al., Immunol. Rev. 63:129-166 (1982), which is incorporated herein by reference).

To exert its biological effects, IL-2 interacts with a specific high-affinity membrane receptor (Greene, W., et al., Progress in Hematology XIV, E. Brown, ed., Grune and Statton, New York (1986), at pgs. 283 ff). The human IL-2 receptor is a complex glycoprotein, with one chain 55kD in size (Leonard, W. et al., J. Biol. Chem. 260:1872 (1985)). A gene encoding this protein has been isolated, and predicts a 272 amino acid peptide, including a 21 amino acid signal peptide, (see, Leonard, W. et al., Nature 311: 626 (1984)).

Much of the elucidation of the human IL-2 receptor's structure and function is due to the development of specifically reactive monoclonal antibodies. In particular, one monoclonal antibody, known as anti-Tac, (Uchiyama et al., J. Immunol. 126:1393 (1981)) has shown that IL-2 receptors can be detected on T-cells, but also on cells of the monocyte-macrophage family, Kupffer cells of the liver, Langerhans' cells of the skin and, of course, activated T-cells. Importantly, resting T-cells, B-cells or circulating machrophages typically do not display the IL-2 receptor (Herrmann, et al., J. Exp. Med. 162:1111 (1985)).

The anti-Tac monoclonal antibody has also been used to define lymphocyte functions that require IL-2 interaction, and has been shown to inhibit various T-cell functions, including the generation of cytotoxic and suppressor T lymphocytes in cell culture. Also, based on studies with anti-Tac, a variety of disorders are now associated with improper IL-2 receptor expression by T-cells, in particular adult T-cell leukemia.

More recently, the IL-2 receptor has been shown to be an ideal target for novel therapeutic approaches to T-cell mediated diseases. The anti-Tac monoclonal antibody can be used either alone or as an immunoconjugate (e.g., with Ricin A, isotopes and the like) to effectively remove cells bearing the IL-2 receptor. These agents can, for example, theoretically eliminate IL-2 receptor-expressing leukemic cells, certain B-cells, or activated T-cells involved in a disease state, yet allow the retention of mature normal T-cells and their precursors to ensure the capability of mounting a normal T-cell immune response as needed. In general, other T-cell specific agents can destroy essentially all peripheral T-cells, which limits therapeutic efficacy. Overall, the use of monoclonal antibodies specific for the IL-2 receptor can be expected to have therapeutic utility in autoimmune diseases, organ transplantation and any unwanted response by activated T-cells. Indeed, clinical trials have been initiated (see, generally, Waldman, T., Science 232:727-732 (1986), which is incorporated herein by reference).

Unfortunately, the use of the anti-Tac monoclonal antibody has certain drawbacks, particularly in repeated therapeutic regimens. As a mouse monoclonal, it does not fix human complement well, whereas a human equivalent may be more efficient.

More importantly, however, anti-Tac monoclonal antibody contains substantial murine amino acid sequences that will be antigenic when injected into a human patient. Numerous studies have shown that the immune response elicited by a patient against the nonbinding portion of an injected mouse monoclonal antibody can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. As increasing numbers of different mouse monoclonal antibodies can be expected to be developed to treat various diseases, after the first and second treatments with different mouse monoclonal antibodies, subsequent treatments can be dangerous in themselves.

Thus, there is a need for improved forms of the anti-Tac monoclonal antibody that are substantially less antigenic, yet easily and economically produced in a manner suitable for therapeutic formulation. The present invention fulfills these needs.

Summary of the Invention

The present invention provides novel compositions useful in the treatment of T-cell mediated human disorders, the compositions containing a chimeric antibody specifically capable of binding to human IL-2 receptors, such as at the epitope bound by the anti-Tac monoclonal antibody. The IL-2 chimeric antibody can have two pairs of light chain/heavy chain complexes, wherein at least one pair has chains comprising mouse variable regions joined with human constant region segments, with or without naturally-associated J and D segments.

The chimeric antibodies, or binding fragments thereof, of the present invention may be produced by a variety of recombinant DNA techniques, with ultimate expression in transfected cells, preferably immortalized

eukaryotic cells, such as myeloma or hybridoma cells. Polynucleotides comprising a first sequence coding for a human immunoglobulin constant region and a second sequence coding for the desired mouse immunoglobulin variable or hypervariable region can be produced synthetically or by combining appropriate cDNA and genomic DNA segments.

When the chimeric antibodies are complexed with a cytotoxic agent, such as a radionuclide, a ribosomal inhibiting protein or a cytotoxic agent active at cell surfaces, the compounds will be particularly useful in treating T-cell mediated disorders. These compounds can be provided in a pharmaceutically accepted dosage form, which will vary depending on the mode of administration.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the DNA coding sequence and putative amino acid sequence of the V and J regions of the anti-Tac light chain.

Figure 2 depicts the DNA coding sequence and putative amino acid sequence of the V and J regions of the anti-Tac heavy chain.

Figures 3-10 are schematic diagrams of the plasmids utilized to demonstrate the present invention.

Figure 11 represents an overview of a preferred strategy for preparation of V and J regions for insertion into plasmids.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, DNA sequences encoding mouse variable/human constant region chimeric antibodies capable of binding epitopes on human IL-2 receptors are provided. When placed in expression vectors and suitable hosts, large quantities of chimeric antibodies can be produced. Preferably, the chimeric antibodies will have substantially the same binding profile or characteristics as (e.g., be cross-reactive or capable of blocking) the binding of the anti-Tac monoclonal antibody, such as antibodies produced by the myeloma cell line deposited with the A.T.C.C. and designated accession number CRL 9688. These chimeric antibodies find use, for example, in the treatment of T-cell mediated disorders in human patients.

The basic immunoglobin structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25kD) and one "heavy" chain (about 50-70kD). The NH_2-terminus of each chain begins a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The COOH terminus of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda. Heavy chains are classified (and subclassified) as gamma, mu, alpha, delta, or epsilon, and define the immunoglobulin's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 12 more amino acids. (See, generally, Fundamental Immunology, Ed. Paul, W. Chapter 7, pgs. 131-166, Raven Press, N.Y. (1984), which is incorporated herein by reference.)

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to

different species. For example, the variable (V) segments of the genes for a mouse monoclonal antibody may be joined to human constant (C) segments, such as γ_1, and γ_3. A preferred therapeutic chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a human antibody, although other mammalian species may be used.

Human chimeric antibodies have at least three potential advantages over mouse antibodies for use in human therapy:

1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the C region of the chimeric antibody as foreign, and therefore the antibody response against an injected chimeric antibody should be less than against a totally foreign mouse antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (Shaw, D. et al., J. Immunol. 138:4534-4538 (1987)). It is possible that injected chimeric antibodies will have a half-life more like that of human antibodies, allowing smaller and less frequent doses to be given.

In one aspect, the present invention is directed to recombinant DNA segments encoding the heavy and/or light chain variable or hypervariable regions from the anti-Tac monoclonal antibody. The DNA segments encoding these regions will typically be joined to DNA segments encoding appropriate constant regions, such as human gamma heavy chain regions or human kappa light chain regions. The preferred variable region DNA sequences, which on expression code for the

polypeptide chains comprising the anti-Tac light and heavy chain variable regions (with naturally-associated J regions), are shown in Figures 1 and 2, respectively. Due to codon degeneracy and non-critical amino-acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below.

The DNA segments will typically further include an expression control DNA sequence operably linked to the chimeric antibody coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact chimeric antibodies may follow.

It is well known that native forms of "mature" immunoglobulins will vary somewhat in terms of length by deletions, substitutions, insertions or additions of one or more amino acids in the sequences. Thus, both the variable and constant regions are subject to substantial natural modification, yet are "substantially identical" and still capable of retaining their respective activities. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells. Suitable source cells for the DNA sequences and host cells for expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to these naturally-occurring forms of immunoglobulin chains, "substantially identical" modified heavy and light chains can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the

chains can vary from the naturally-occurring sequence at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Alternatively, polypeptide fragments comprising only a portion (usually at least about 60-80%, typically 90-95%) of the primary structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity), while exhibiting lower immunogenicity. In particular, it is noted that like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities. These may be fused to functional regions from other genes (e.g., enzymes, see, commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene 8:81-97 (1979); Roberts, S. et al, Nature 328:731-734 (1987); and U.S. Patent no. 4,703,008, all of which are incorporated herein by reference). Preferred DNA segments encoding variable regions of the present invention will typically be substantially homologous to the sequences of Figures 1 and 2 (i.e., capable of hybridizing to the sequences under stringent conditions of low salt and high temperature), most preferrably at least about 90-95% homologous or more.

The nucleic acid sequences of the present invention capable of ultimately expressing the desired chimeric antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production, but cDNA sequences may also be utilized (see, European Patent Application Nos. 85102655.8, 85305604.2, 84302368.0 and 85115311.4, as well as PCT Application Nos. GB85/00392 and US86/02269, all of which are incorporated herein by

reference).

As stated previously, the DNA sequences of the present invention (typcially at least about 30 contiguous nucleotides encoding 10 amino acids from the sequences in Figures 1 and 2) will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

E. coli is one prokaryotic host useful particularly for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast may also be used for expression. Saccharomyces is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to produce the polypeptides of

the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen, C. et al., Immunol. Rev. 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment may be used for other cellular hosts. (See, generally, Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole chimeric antibodies, their dimers, or individual light and heavy chains of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, fraction column chromatography, gel electrophoresis and the like. (See, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982).) Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological

Methods, Vols. I and II, Eds. Lefkovits and Pernis, Academic Press, New York, N.Y. (1979 and 1981).)

The chimeric antibodies of the present invention will typically find use individually in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as IL-2 receptor bearing, then the chimeric antibodies are suitable (see, U.S.S.N. 7-085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference).

For example, typical disease states suitable for treatment graft versus host disease and most patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include Type I diabetes, multiple sclerosis, rheumatoid arthritis, Lupus erythematosus, and Myasthenia Gravis.

The antibodies of the present invention may also be used in combination with other antibodies, particularly human chimeric antibodies or human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Eds. Bernard, et al., Springer-Verlag, N.Y. (1984), which is incorporated herein by reference.

The chimeric antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include a cephalosporin or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, sulfa drugs, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject chimeric antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when

attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, such as a chimeric antibody, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatinum; and cytotoxic proteins such as ribosomal inhibiting proteins, pokeweed antiviral protein, abrin and ricin (or their A-chains, diphtheria toxin A-chains, Pseudomonas exotoxin A, etc.) or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). (See generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), both of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the chimeric antibodies of the present invention. Intact chimeric immunoglobulins or their binding fragments, such as Fab, $F(ab_2)$, etc., are preferably used. Typically, the chimeric antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The chimeric antibodies and pharmaceutical

compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pennsylvania (1980), which is incorporated herein by reference.

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed. It will be

appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human chimeric antibodies or a cocktail thereof can be administered for the prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already, in an amount sufficient to cure or at least partially arrest the infection and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of this invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human chimeric antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

Chimeric antibodies of the present invention can further find a wide variety of utilities in vitro. By way of example, the chimeric antibodies can be utilized for T-cell typing, for isolating specific IL-2 receptor bearing cells or fragments of the receptor, for vaccine preparation, or the like.

For diagnostic purposes, the chimeric antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the chimeric antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the chimeric antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluorescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, the subject antibody composition of the present invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody

concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following examples are offered by way of illustration, not by limitation.

EXPERIMENTAL

Construction of Plasmids

1. Construction began with the plasmid pKcatH described in the literature (Garcia, J.V. et al., Nature 332:383-385 (1986); Fig. 3). This plasmid has the following parts; clockwise:

   a. A 700 bp fragment containing the mouse immunoglobulin heavy chain enhancer (E_H in the Fig. 3).

   b. An 1100 base pair (bp) fragment from the mouse immunoglobulin light chain kappa gene MOPC 41, containing its promoter. There is a Bgl II site 25 bp after the transcription startsite of this promoter.

   c. The bacterial CAT gene (800 bp).

   d. A splice and polyadenylation signal from the animal virus SV40 (850 bp).

   e. Another part of SV40 containing its origin of replication (700 bp);

   f. Part of the plasmid pBR322, extending from the Sph I site to the Eco R1 site of the plasmid pML1 (Lusky, M and Botchan, M. Nature 293:79-81; 2300 bp) including the Amp gene and origin of replication.

2. pKcatH was cut with Bgl II, and the ends filled in with Klenow polymerase. An Xba I linker, having the sequence GCTCTAGAGC, was inserted at the filled-in Bgl II site. The resulting plasmid is called pKcatH - Xba.

3. pKcatH - Xba was cut partially with Xho II, run on an agarose gel, and full-length linear plasmid (i.e., plasmid cut exactly once with Xho II) was isolated. The DNA was cut with Bam HI, run on a gel and the 5600 bp fragment isolated. Because of its size, this fragment had to extend from the Bam site to the Xho II site at the end of the CAT gene (Fig. 3). The fragment was

ligated by itself. The resulting plasmid is called pKcatH - Xba - BX. It is similar to pKcatH but has an Xba I site in place of the Bgl II site and is missing the Xho II - Bam HI SV40 fragment (Fig. 4).

4. pKcatH - Xba - BX was cut partially with Eco RI, run on an agarose gel, and full-length linear plasmid was isolated. This DNA was cut with Bam HI, and the 2600 bp fragment isolated. This fragment extended from the Eco R1 site before E_H to the Bam HI site. The plasmid pSV2gpt, described in the literature (Mulligan, R.C. and Berg, P., Proc. Nat. Acad. Sci. USA 78:2072-2976 (1980)), was cut with Eco R1 and Bam HI and the large Eco RI - Bam HI fragment ligated to the Eco RI - Bam HI fragment from pKcatH - Xba - BX. The resulting plasmid is called pSV2gpt - E_H - κ, (Fig. 5).

5. The plasmid pSV2neo (Southern, P.J. and Berg, P. J. Mol. App. Genet. 1:327-341 (1982)), was cut with Eco RI and Bam HI, and the large Eco RI - Bam HI fragment ligated to the same Eco RI - Bam HI fragment from pKcatH - Xba - BX used in (4). The resulting plasmid is called pSV2neo - EH - κ, which is like pSV2gpt - EH - κ, but has the Neo gene in place of the Gpt gene.

6. pSV2gpt - E_H - κ was cut with Xba I and Bam HI and the ends filled in with Klenow polymerase. A fragment of the cloned human κ constant segment gene (Hieter, P.A. et al., Cell 22:197-207 (1980)) was purified, extending from a Hind III site 336 bp before the coding region, to an Xba I site about 800 bp beyond the coding region, and the ends filled in. The two fragments were ligated together, and a plasmid selected in which the Hind III site of the second fragment was joined to the Xba I site and the Xba I site of the second fragment to the Bam HI site. Because of the sequences of these sites, this recreated an Xba I site and a Bam HI site. The new plasmid is called pVκ1 (Fig. 6).

7. An Xba I fragment containing the VJ region of the cloned anti-Tac light chain gene was prepared by in vitro mutagenesis (see, below). pVκ1 was cut with Xba I, treated with phosphatase, and ligated with the Xba I fragment. A plasmid was selected in which the VJ region had the same orientation as the following C region, and called pLTAC2 (Fig. 7).

8. pSV2neo - E_H - κ was cut with Xba I and Bam HI and the ends filled in with Klenow polymerase. A 2800 bp fragment containing the human Cγ1 gene was purified from the phage HG3A (Ellison, J.W. et al., Nucleic Acids Res. 10:4071-4079 (1982)), extending from a Hind III site 210 bp before the CH1 exon to a Pvu II site about 1100 bp after the CH3 exon, and the ends filled in. The two fragments were ligated together, and a plasmid selected in which the Hind III site of the second fragment was joined to the Xba I site and the Pvu II site of the second fragment to the Bam HI site. Because of the sequences of these sites, this recreated an Xba I site and a Bam HI site. The new plasmid is called pVγ1neo (Fig. 8).

9. pSV2neo - E_H - κ was cut with Xba I and Bam HI and the ends filled in with Klenow polymerase. A 3600 bp fragment containing the human Cγ3 gene (Takahashi, N. et al., Cell 29:671-679 (1982)) was purified extending from a Hind III site 210 bp before the CH1 exon to a Pvu II site about 1100 bp after the CH3 exon, and the ends filled in. The two fragments were ligated together, and a plasmid selected in which the Hind III site of the second fragment was joined to the Xba I site and the Pvu II site of the second fragment to the Bam HI site. Because of the sequences of these sites, this recreated an Xba I site and a Bam HI site. The new plasmid is called pVγ3neo and is identical to pVγ1neo except it has the Cγ3 gene instead of Cγ1.

10. An Xba I fragment containing the VJ region of the cloned anti-Tac heavy china gene was prepared by in vitro mutagenesis (see, below). pVγ1neo was cut with Xba I, treated with phosphatase, and ligated with the Xba I fragment. A plasmid was selected in which the VJ region had the same orientation as the following C region, and called pHTAC.

11. Three additional plasmids were prepared respectively from pVγ1neo, pVγ3neo and pHTAC, called respectively pVγ1, pVγ3 and pGTAC1. In each case, the original plasmid was cut with Hind III and Bam HI, and the large Hind III - Bam HI fragment purified. The plasmid pXBohph containing the Hyg gene (Blochlinger, K. and Diggelmann, H. Mol. Cell. Biol. 4:2929-2931 (1984)) was cut with Hind III and Bam HI and a 1600 bp fragment containing the Hyg gene purified. The fragments from the original plasmids were each ligated to the pXbohph fragment. pVγ1 is shown in Fig. 9 and pGTAC1 in Fig. 10.

12. pHTAC was cut with Xba I and the small Xba I fragment, containing the heavy VJ region, purified. pVγ3 was cut with Xba I, treated with phosphatase, and ligated with the small Xba I fragment. A plasmid was selected in which the VJ region had the same orientation as the following C region, and called pGTAC3. It is similar to pGTAC1, but has the Cγ3 region instead of the Cγ1 region.

Cloning of Light and Heavy Chain cDNA.

mRNA was extracted from approximately 10-8 ascites cells of the anti-Tac hybridoma by the guanidium isothiocyanate method followed with poly A selection on Hybond-mAP paper (Amersham). cDNA was prepared by the method of Gubler and Hoffman (Gubler, U. and Hoffman, B.J. Gene 25:263-269 (1983)), treated with Eco R1 methylase, ligated to Eco RI linkers, cloned into λgt10 arms (Promega Biotec), packaged (Strategene packaging extract) and plated on C600Hfl cells. Oligonucleotides respectively 43 and 37 nucleotides long that hybridized to the 5' ends of the mouse Cκ and Cγ1 segments were synthesized (Applied Biosystems Model 380B DNA synthesizer). The oligonucleotides were 5' end-labelled and used to screen the plaques (Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference).

Approximately 10,000 plaques were screened with the oligonucleotide probes. About 100 plaques were positive for the γ probe and 40 for the γ probe.

The cDNA inserts from 4 κ positive and 4 γ2a positive phage were subcloned into the Eco R1 sites of pUC19 and M13mp19. Partial sequencing by the dideoxy method (Sanger, F. et al., Proc. Nat. Acad. Sci. USA 74:5463-5467 (1977)) showed that two of the κ isolates had one sequence, and the other two had another sequence. In one pair, a κ V gene segment was joined to the JK2 segment out of its reading frame. In addition, the conserved Cys at position 23 was absent in this V segment, and the sequences of the two isolates differed slightly. Presumably, these clones were the result of an aberrant joining event in one κ allele, which continued to undergo somatic mutation after the formation of the hybridoma.

The VJ segments of the other pair of κ clones were sequences completely and were identical (Fig. 1). This light chain uses the JK5 segment and contains all the amino acid residues conserved in mouse κ light chains (Kabat, E. A. et al., Sequences of Proteins of Immunological Interest, particularly pg. 45 et seq, all of which is incorporated

herein by reference).

Partial sequencing of the four γ2a clones showed they were from the same gene. The VJ segments of two were sequenced completely and had no differences. This heavy chain (Fig. 2) uses the JH2 segment and is of subgroup II (Kabat, E.A. et al., supra, at pgs. 121-127). The sequence is somewhat unusual because it contains no detectable D segment and has an N region containing 10 Gs. As both alleles of the κ light chain were accounted for and only one heavy chain sequence was detected, we tentatively assigned these sequences to the anti-Tac antibody genes.

Preparation of the Chimeric Genes

Plasmid vectors were prepared for the construction and expression of chimeric light and heavy chain genes as described above. The plasmid pVκ1 (Fig. 6) contains the human κ genomic C segment, including 336 bp of the preceding intron and the poly A signal. It also contains a strong promoter sequence from the MOPC 41 κ gene, and the heavy chain enhancer sequence. There is a unique Xba I site between the promoter and the C intron. The plasmid also contains the gpt gene for selection. Two other very similar plasmids were prepared by using the human γ1 and γ3 C regions in place of the κ C region. In each case, the region inserted between the Xba I and Bam HI sites extended form about 210 bp 5' of the CH1 exon to about 1100 bp 3' of the CH3 exon. In addition, the gpt gene was replaced with the hyg gene to confer resistance to hygromycin.

Our strategy was to insert the VJ region from the anti-Tac κ cDNA, followed by a splice donor signal, at the Xba I site of pVκ1. Doing so would create a chimeric κ gene, with a synthetic intron between the mouse VJ and human Cκ segments. For this purpose, we devised a form of primer-directed mutagenesis (Fig. 11). The κ Eco R1 cDNA clone was moved into a variant of M13mp11, in which the Xba 1 and Eco R1 sites in the polylinker were abuting. An oligonucleotide was synthesized, of which the first 22 residues hybridized to the last 22 bp of the JK5 segment. The next 16 nucleotides

were the same as the sequence that follows JK5 in mouse genomic DNA, and therefore included a splice donor signal. The final nucleotides of the oligo consisted of an Xba I site followed by a short irrelevant sequence.

This oligonucleotide was hybridized to the M13 phage DNA containing the κ cDNA and extended with Klenow polymerase (Fig. 3). The DNA was denatured and hybridized to a "reverse primer," which represents M13 DNA 5' to the cDNA insert. The reverse primer was extended, and the DNA cut with Xba I. The Xba I fragment consisting of the extended VJ segment of the cDNA was purified, and cloned in the correction orientation into the Xba I site of pVκ1 to obtain the plasmid pLTAC2 (Fig. 7). Hence, the final chimeric κ gene has a VJ-C intron, of which the first 14 bp and last 209 bp are respectively the same as in mouse and human genomic DNA, as verified by direct sequencing of the construct. The gene is transcribed from a κ promoter, stimulated by the heavy chain enhancer. Based on results obtained with deleted introns, we expected that the intron would be correctly spliced from the transcribed RNA.

In an analogous manner, the VJ region from the anti-Tac γ2a heavy chain cDNA, followed by a splice donor signal, was inserted into the Xba I site of pVγ1neo. The resulting plasmid pHTAC contains a chimeric heavy chain gene, with a synthetic intron between the mouse VJ and human Cγ1 segments. The additional plasmids pGTAC1 and pGTAC3, containing the Hyg gene and chimeric heavy genes with the human Cγ1 Cγ3 constant regions respectively, were constructed as described above (Fig. 10).

Details of Insertion of VJ cDNA Regions Into Plasmids

RF DNA of the phage M13mp11 was cut with Eco R1 and Xba I, the ends were filled-in, and the DNA ligated was ligated and transformed into JM101 cells. A plaque was picked and the DNA was sequenced to verify that the ends of the DNA had joined correctly, recreating the Eco R1 and Xba I sites with the intervening DNA segment deleted. This phage is designated M13mp11D. Eco R1 fragments containing the anti-Tac light and heavy chain cDNAs were separately cloned into the Eco R1 site of M13mp11, so that their 5' ends abuted the Xba I site. The resulting phage are respectively denoted M13mp11L and M13mp11H.

The following 48-nucleotide long primer was synthesized and gel-purified: CCAGAATTCTAGAAAAGTGTACTTACGTTTCAGCTCCAGCTTGGTCCC. From the 3' end, the first 22 residues of the primer are the same as the last 22 bp of the JK5 segment (non-coding strand). The next 16 nucleotides are the same as the sequence that follows JK5 in mouse genomic DNA and therefore includes a splice donor signal (abbreviated SD in Fig. 11). The final nucleotides of the oligo consist of an Xba I site followed by a short irrelevant sequence. Approximately 1 ug of single-stranded M13mp11L DNA was mixed with 50 ng of primer in 35 ul of 10 mM Tris, pH 7.4, 60 mM NaCl, 10 mM $MgCl_2$ and incubated for 15 min at 50'C and then 15 min at 23'C. 4 ul of a solution of 200 uM each dNTP was added, together with 5u Klenow polymerase. The solution was incubated for 30 minutes at 37'C. 50 ng of the "reverse primer" AACAGCTATGACCATG (New England Biolabs), which can hybridize to the newly synthesized strand upstream of the Xba I site (Fig. 11), was added. The solution was incubated at 95'C for 3 min and put on ice. An additional 4 ul of 200 uM each dNTP and 5u Klenow polymerase was added, and the solution incubated for 30 minutes at 37'C. The solution was extracted with phenol-chloroform, precipitated with ethanol, resuspended, and digested with 20u Xba I. The digested DNA was run on a 4% polyacrylamide gel and visualized with ethidium bromide. In addition to high molecular weight DNA, an approximately 400

bp fragment was visible, corresponding to the VJ region of the light chain cDNA with a "tail" (Fig. 11). The fragment was cloned directly into the Xba I site of pVκ1 in the correct orientation.

The following 50-nucleotide long primer was synthesized and gel-purified: CCAGAATTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGAGAGTGG. From the 3' end, the first 21 residues of the primer are the same as the last 21 bp of the JH2 segment (non-coding strand). The next 19 nucleotides are the same as the sequence that follows JH2 in mouse genomic DNA and therefore includes a splice donor signal. The final nucleotides of the oligo consist of an Xba I site followed by a short irrelevant sequence. This primer was hybridized to M13mp11H DNA following the same protocol as above, in order to synthesize a fragment containing the VJ region of the heavy chain cDNA. The fragment was cloned directly into the Xba I site of pVγ1neo in the correct orientation.

Chimeric Antibody Specificity

CR2-2 and CEM cells are human T cell lines that are respectively positive and negative for surface expression of the IL2 receptor (IL2R). Whole CR2-2 cells were used to demonstrate binding specificity of the chimeric antibody in an ELISA assay, and CEM cells were used as a negative control.

Antibody to be tested was prepared in several ways. Anti-Tac monoclonal antibody, supplied by T. Waldmann, was purified from mouse ascites by passage through a DEAE-dextran column. The cell line L40H4, created by transfecting SP2/0 cells with the chimeric light and gamma 1 heavy chain plasmids pLTAC2 and pGTAC1 (described above), was injected into mice to form an ascites. From 3 mls of ascites fluid, 650 μg of chimeric gamma 1 antibody was purified by passage through a column of Baker Abx and a size exclusion column. Also, to detect antibody production in tissue culture, 10^6 cells each of L40H4 and the cell lines 51.3 and L40H2 (which had been created by transfecting SP2/0 with pLTAC2 and the

gamma 3 heavy chain plasmid pGTAC3 described before) and the non-producing line Sp2/0 itself, were plated in 1 ml of DME medium each. The media supernatent, containing any secreted chimeric antibodies, was harvested after 24 hr. and used directly in the assay,

Cultured cells of each type were washed in FACS buffer (0.1% BSA, .01% sodium azide in Dulbecco's phosphate buffered saline). 2 x 10^6 cells were mixed with either purified antibody in 20 µl FACS buffer or 20 µl media supernatent from the transfected cell lines, and incubated on ice for 2 hr. The cells were washed 3 times with 1 µl of FACS buffer (being collected by brief centrifugation after each wash). The cells that had been incubated with anti-Tac antibody (or no antibody as a control) were mixed with 0.5 µl peroxidase-conjugated goat anti-mouse antibody $(Fab)'_2$ fragment (Tago Immunologicals, Burlingame, California) in 20 µl FACS buffer, and the cells that had been incubated with chimeric antibody were mixed with 0.5 µl peroxidase-conjugated goat anti-human gamma chain $(Fab)'_2$ fragment (Tago) in 20 µl FACS buffer. The cells were incubated for 30 min on ice and then washed 3 times with FACS buffer. They were then mixed with 100 µl peroxidase development solution and incubated for 5 min at room temperature. The cells were spun out and the supernatents were transferred to a 96-well plate and the OD's determined in an ELISA reader. The OD's at 414 nm for the cells treated with each antibody are given in Table 1.

As expected the anti-Tac antibody itself bound to the IL2R+ CR2-2 cells but not to the IL2R- CEM cells. An equal amount of purified gamma 1 chimeric antibody gave an equivalent amount of binding to the CR2-2 cells as the anti-Tac, and also failed to bind to the CEM cells. All the supernatants from both the chimeric antibody producing cells bound to the CR2-2 cells. As additional negative controls, the 51.3 supernatant did not bind to the CEM cells, and supernatant from the parental SP2/0 cells did not bind the CR2-2 cells.

In combination with the derivation of the light and

heavy chain genes from the anti-Tac hybridoma, these results show that the chimeric antibodies retained specificity for the human IL2R.

TABLE 1

Whole-Cell ELISA Assay of Chimeric Antibodies

	CR2-2 cells (IL2R +)	CEM cells (IL2R -)
Mouse Gamma 2a		
none	.037	-
anti-Tac (100ng)	.679	.024
Human Gamma 1		
none	.084	-
purified ascites (100ng)	.641	.044
L40H4 (20 µl sup.)	.481	-
Human Gamma 3		
51.3 (20 µl sup.)	.381	.038
L40H2 (20 µl sup.)	.932	-

To quantitate the rate of secretion of the chimeric antibodies, an ELISA assay was performed, using standard methods. The results are presented in Table 2. About 10^6 cells each of the chimeric gamma 1 secreting line L40H0 and the chimeric gamma 3 secreting line 51.3 were plated in 1 ml of DME medium with 5% fetal calf serum, and the supernatents collected after 24 hr. A 96-well plate was coated with goat anti-human antibodies (Tago Immunologicals). Different wells were incubated with increasing known amounts of gamma 1 chimeric antibody (purified from ascites) and with 1 μl of the chimeric antibody supernatents. Briefly, the plate was washed, incubated with peroxidase-conjugated goat anti-human gamma chain antibody, washed again, incubated with peroxidase developing solution and the OD's at 414 nm determined in an ELISA reader. By comparison with the standard curve from the purified antibody, the media supernatants contained respectively about 8 and 7 ng per μl, that is the cells secreted 8 and 7 μg antibody per 10^6 cells per 24 hr.

TABLE 2

Rate of Chimeric Antibody Secretion

	OD_{414}
Purified gamma 1 chimeric	
3 ng	.426
4 ng	.535
6 ng	.734
8 ng	1.004
L40H4 (1 μl sup.)	1.014
51.3 (1 μl sup.)	.845

The mixed lymphocyte reaction is a model for transplant rejection and was used to analyze the effectiveness of the chimeric anti-Tac antibodies. The experiments shown in Table 3 below were performed by standard methods (see, e.g., Strong, D.M. et al., In Vitro Stimulation of Murine Spleen Cells Using a Microculture System and a Multiple Automated Sample Harvester, J. Immunol. Meth., 2:279 (1973)). Briefly, human peripheral blood lymphocytes from two unrelated human donors were purified. The lymphocytes from the two donors were incubated in wells of a 96-well plate, either separately, mixed together, or mixed together with 1 μg/ml of the indicated antibody added each day. When mixed together, the T-cells recognize each other as foreign, as T cells recognize an organ transplant as foreign. They therefore proliferate, as measured by uptake of 3H-labeled thymidine after three days. The numbers in Table 3 are the average thymidine uptake in cpm from triplicate wells ± the standard deviation. Addition of either the original anti-Tac antibody or either of the chimeric antibodies strongly inhibits proliferation of the cells (percent inhibition shown in parentheses), by binding to the IL2R. This prevents IL2 from binding to the IL2R, which is required for T-cell proliferation. These experiments show that the chimeric antibody can be used to reduce the response of T-cells to foreign cells and may therefore have medical applications in preventing transplant rejection.

TABLE 3

Anti-IL2R Antibody Inhibition of Mixed Lymphocyte Reaction

	Exp. 1	Exp. 2
Donor A	510 ± 292	151 ± 5
Donor B	901 ± 38	712 ± 29
A + B	11740 ± 1593	19806 ± 2034
A + B + anti-Tac	4262 ± 346 (73%)	7311 ± 461 (66%)
A + B + Human Gamma 1	4126 ± 370 (74%)	6721 ± 981 (69%)
A + B + Human Gamma 3	-	6423 ± 423 (71%)

From the foregoing, it will be appreciated that the chimeric antibodies of the present invention offer numerous advantages of other human IL-2 receptor-specific antibodies. In comparison to anti-Tac mouse monoclonal antibodies, the present human chimeric antibodies can be more economically produced and contain substantially less foreign amino acid sequences. This reduced likelihood of antigenicity after injection into a human patient represents a significant therapeutic improvement.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

CLAIMS

1. A method of treating T-cell mediated disorders in a human patient, said method comprising administering to said patient a therapeutically effective dose of a mouse variable/human constant region chimeric antibody composition specifically reactive with IL-2 receptors on human T-cells.

2. A method according to Claim 1, wherein the mouse variable region comprises the entire variable region from heavy and light chains of an anti-Tac monoclonal antibody as produced by the cell line designated A.T.C.C. Accession No. CRL 9688.

3. A method according to Claim 1, wherein at least one of the mouse variable regions is joined with a naturally-associated mouse J segment.

4. A method according to Claim 1, wherein the human light chain constant region comprises a κ chain constant region.

5. A method according to Claim 1, wherein the human light chain constant region comprises a γ_1 or γ_3 chain constant region.

6. A method according to Claim 1, wherein the composition comprises the chimeric antibody complexed with a cytotoxic agent.

7. A method according to Claim 1, wherein the cytotoxic agent is a ribosomal inhibiting protein, a radionuclide or cytotoxic agent active at cell surfaces.

8. A chimeric antibody capable of binding to a human IL-2 receptor epitope, said antibody having two pairs of light chain/heavy chain complexes, wherein at least one pair has chains comprising mouse variable region and human constant region segments.

9. A chimeric antibody according to Claim 8, wherein the mouse variable region is adjacent to a naturally associated J segment.

10. A chimeric antibody according to Claim 8, which inhibits the binding of monoclonal antibodies secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.

11. A chimeric antibody according to Claim 10, wherein at least one variable region comprises the amino acid sequence of a variable region of the anti-Tac monoclonal antibody.

12. A chimeric antibody according to Claim 8, which was produced in a myeloma or hybridoma cell.

13. A chimeric antibody according to Claim 8, which was expressed in a myeloma cell from a transfected DNA sequence comprising a mouse cDNA segment joined to a human genomic DNA segment.

14. A chimeric antibody according to Claim 8 complexed with a cytotoxic agent or signal agent.

15. A heavy immunoglobulin chain comprising a human heavy chain constant region and a variable chain region which is substantially identical to a monoclonal antibody heavy chain variable region secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.

16. A heavy immunoglobulin chain according to Claim 15, wherein the variable and constant region are joined by a mouse J segment.

17. A light immunoglobulin chain comprising a human light chain constant region and a variable chain region which is substantially identical to a monoclonal antibody light chain variable region secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.

18. A polynucleotide molecule comprising a first sequence coding for a human immunoglobulin constant region and a second sequence coding for a mouse immunoglobulin variable region, wherein said second sequence encodes substantially for one of the amino acid sequences of Figures 1 or 2.

19. A cell line transfected with a polynucleotide of Claim 18.

20. The cell line designated A.T.C.C. Accession No. CRL 9688.

21. A DNA segment encoding a portion of an immunoglobulin light chain, said segment comprising at least about 30 contiguous nucleotides from the sequence of Figure 1.

22. A DNA segment according to Claim 21, wherein the segment encodes a full length light chain variable region substantially homologous to the sequence in Figure 1.

23. A DNA segment encoding a portion of an immunoglobulin heavy chain, said segment comprising at least about 30 contiguous nucleotides from the sequence of Figure 2.

24. A DNA segment according to Claim 23, wherein the segment encodes a full length heavy chain variable region substantially homologous to the sequence in Figure 2.

25. An expression vector comprising a heterologous promoter operably linked to a DNA segment according to Claim 21 or 23.

26. An immortalized cell line transformed with an expression vector according to Claim 25.

27. A protein composition comprising at least about ten contiguous amino acids from the protein sequence of Figure 1 or Figure 2 fused to a heterologous polypeptide.

28. A protein composition according to Claim 27, wherein the heterologous polypeptide is an immunoglobulin constant region.

29. A protein composition according to Claim 27, wherein the protein is glycosylated.

30. A recombinantly produced antibody exhibiting substantially the same antigen binding profile as an immunoglobulin secreted by a cell line designated A.T.C.C. Accession No. CRL 9688.

```
        10         20         30         40         50         60
AAATTCAAAGACAAAATGCATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCC
              MetHisPheGlnValGlnIlePheSerPheLeuLeuIleSerAla

        70         80         90        100        110        120
TCAGTCATAATGTCCAGAGGACAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCA
SerValIleMetSerArgGlyGlnIleValLeuThrGlnSerProAlaIleMetSerAla

       130        140        150        160        170        180
TCTCCAGGGGAGAAGGTCACCATAACCTGCAGTGCCAGCTCAAGTATAAGTTACATGCAC
SerProGlyGluLysValThrIleThrCysSerAlaSerSerSerIleSerTyrMetHis

       190        200        210        220        230        240
TGGTTCCAGCAGAAGCCAGGCACTTCTCCCAAACTCTGGATTTATACCACATCCAACCTG
TrpPheGlnGlnLysProGlyThrSerProLysLeuTrpIleTyrThrThrSerAsnLeu

       250        260        270        280        290        300
GCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGATCTGGGACCTCTTACTCTCTCACA
AlaSerGlyValProAlaArgPheSerGlySerGlySerGlyThrSerTyrSerLeuThr

       310        320        330        340        350        360
ATCAGCCGAATGGAGGCTGAAGATGCTGCCACTTATTACTGCCATCAAAGGAGTACTTAC
IleSerArgMetGluAlaGluAspAlaAlaThrTyrTyrCysHisGlnArgSerThrTyr

       370        380        390        400
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```

FIG._1.

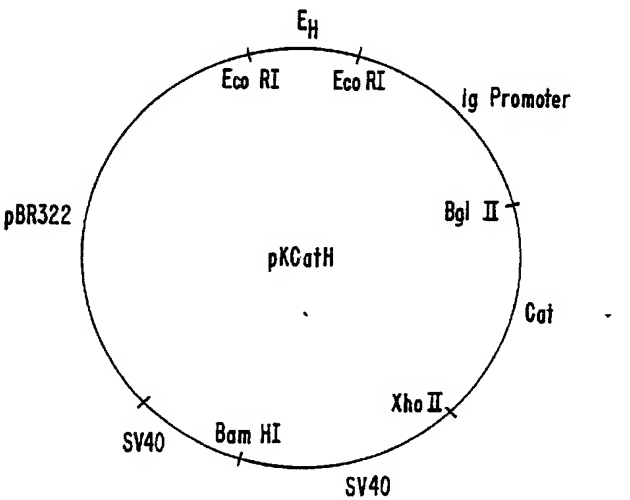

FIG._3.

```
        10         20         30         40         50         60
GCATCCTCTCCACAGACACTGAAAACTCTGACTCACAATGGAAAGGCACTGGATCTTTCT
                                        MetGluArgHisTrpIlePheLe

        70         80         90        100        110        120
CTTCCTGTTTTCAGTAACTGCAGGTGTCCACTCCCAGGTCCAGCTTCAGCAGTCTGGGGC
uPheLeuPheSerValThrAlaGlyValHisSerGlnValGlnLeuGlnGlnSerGlyAl

       130        140        150        160        170        180
TGAACTGGCAAAACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACCTT
aGluLeuAlaLysProGlyAlaSerValLysMetSerCysLysAlaSerGlyTyrThrPh

       190        200        210        220        230        240
TACTAGCTACAGGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGATTGG
eThrSerTyrArgMetHisTrpValLysGlnArgProGlyGlnGlyLeuGluTrpIleGl

       250        260        270        280        290        300
ATATATTAATCCTAGCACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCCAC
yTyrIleAsnProSerThrGlyTyrThrGluTyrAsnGlnLysPheLysAspLysAlaTh

       310        320        330        340        350        360
ATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATTTGA
rLeuThrAlaAspLysSerSerSerThrAlaTyrMetGlnLeuSerSerLeuThrPheGl

       370        380        390        400        410        420
GGACTCTGCAGTCTATTACTGTGCAAGAGGGGGGGGGGTCTTTGACTACTGGGGCCAAGG
uAspSerAlaValTyrTyrCysAlaArgGlyGlyGlyValPheAspTyrTrpGlyGlnGl

       430        440
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yThrThrLeuThrValSerSer
```

FIG._2.

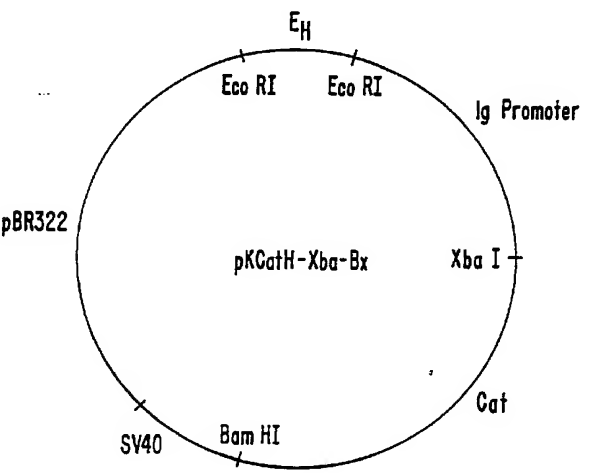
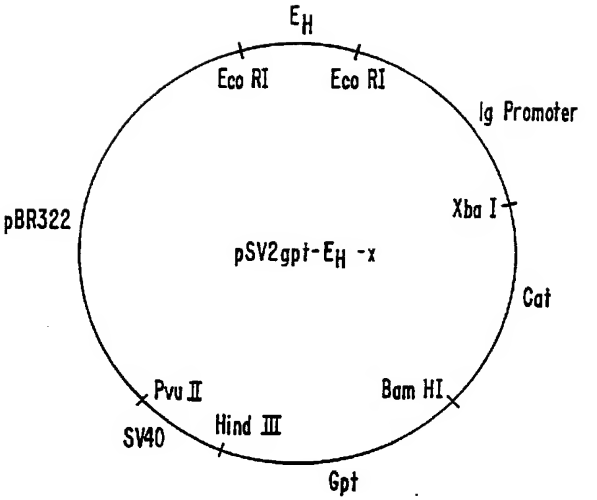


FIG._4.


E_H
Eco RI
Eco RI
Ig Promoter
Xba I
pBR322
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Cat
Pvu II
Bam HI
Hind III
SV40
Gpt

FIG._5.

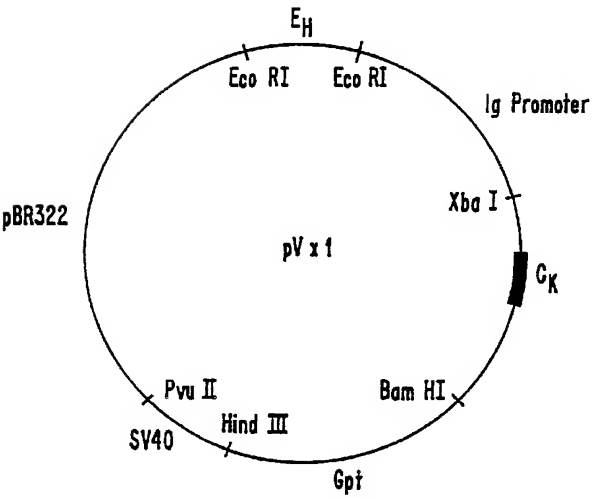
E_H
Eco RI
Eco RI
Ig Promoter
Xba I
pBR322
pV x 1
C_K
Pvu II
Bam HI
Hind III
SV40
Gpt

FIG._6.

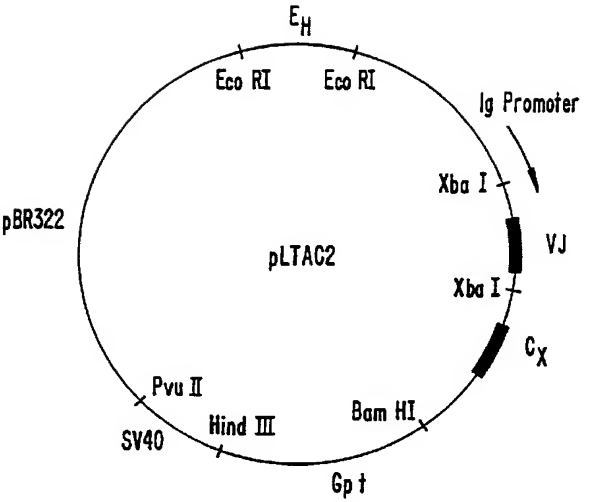
E_H
Eco RI
Eco RI
Ig Promoter
Xba I
pBR322
pLTAC2
VJ
Xba I
C_X
Pvu II
Hind III
Bam HI
SV40
Gp t

FIG._7.

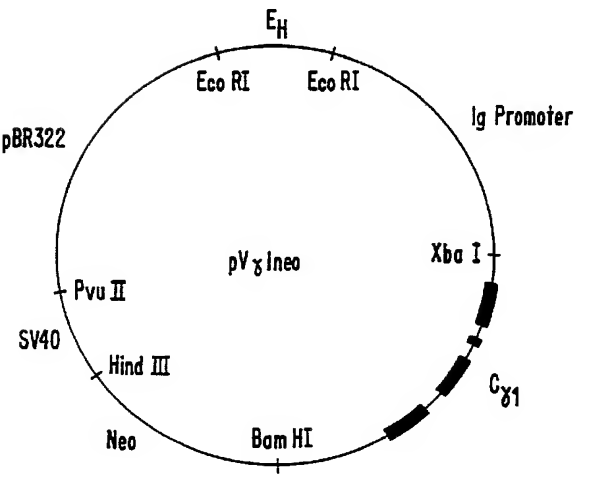
E_H
Eco RI
Eco RI
Ig Promoter
pBR322
pV$_\gamma$1neo
Xba I
Pvu II
SV40
Hind III
$C_{\gamma 1}$
Neo
Bam HI

FIG._8.

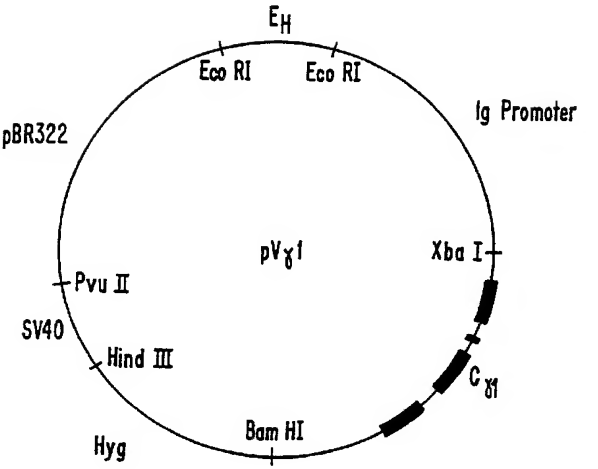
E_H
Eco RI
Eco RI
Ig Promoter
pBR322
$pV_{\gamma}1$
Xba I
Pvu II
SV40
Hind III
$C_{\gamma}1$
Bam HI
Hyg

FIG._9.

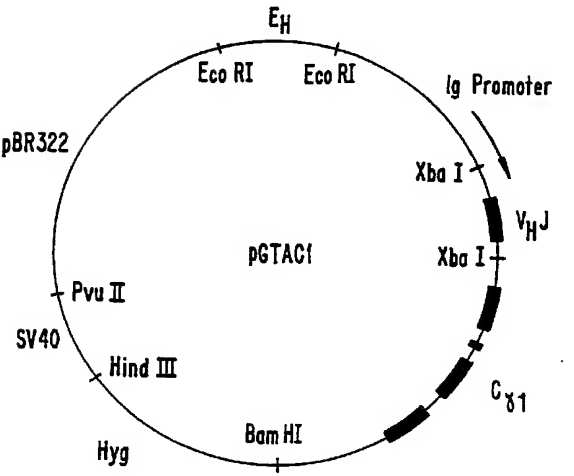
E_H
Eco RI
Eco RI
Ig Promoter
pBR322
Xba I
V_HJ
pGTAC1
Xba I
Pvu II
SV40
Hind III
$C_{\gamma}1$
Bam HI
Hyg

FIG._10.

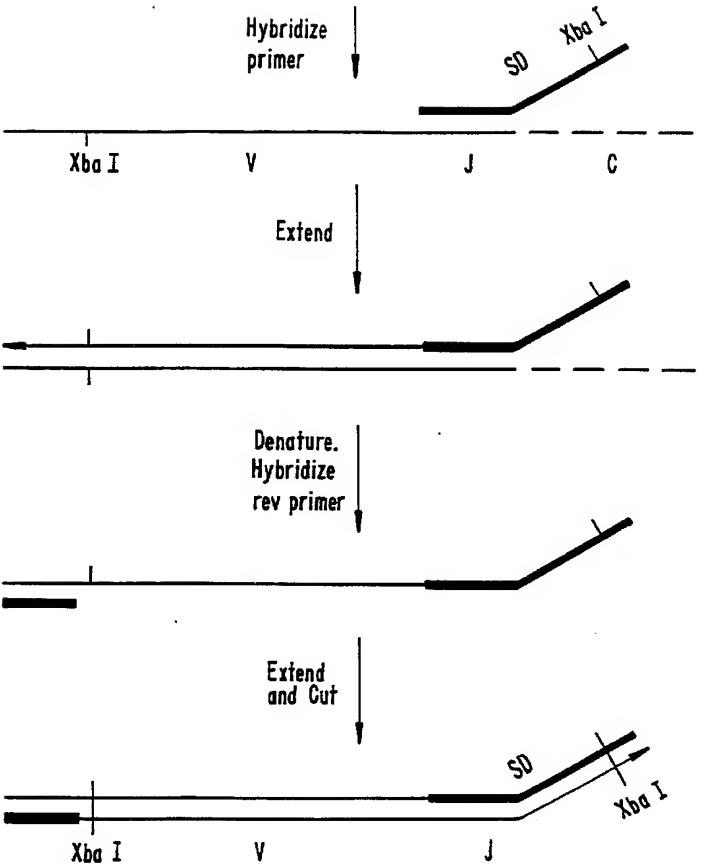
Hybridize primer
SD
Xba I
Xba I
V
J
C
Extend
Denature. Hybridize rev primer
Extend and Cut
SD
Xba I
Xba I
V
J

FIG. 11.

INTERNATIONAL SEARCH REPORT

International Application PCT/US89/01578

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 39/395; C12N 15/00; C12N 5/00; C07K 13/00; C07K 15/04.
US: 424/85.91; 424/85.8; 530/387; 435/68; 435/172.2; 435/240.27; 536/27

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/85.8; 424/85.91; 530/387; 530/388; 530/391; 530/808; 530/821; 530/825; 435/68; 435/70; 435/172.2; 435/172.3; 435/240.27; 435/240.2; 536/27; 935/32; 935/15.

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Computer database searches on APS, Biosis.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,664,911 (UHR et al), 12 May 1987 (12.05.87), see columns 2 to 6.	6,7,14
Y	EP, A, 0125023 (GENENTECH et al), 14 November 1984 (14.11.84), see pages 16-25, 28, 29, and 31 to 43.	1-30
Y	EP,A, 0173494 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY), 05 March 1986 (05.03.86), see pages 4-6 and 18-20.	1-30
Y	EP,A,0184187 (TEIJIN LIMITED), 11 June 1986 (11.06.86), see pages 8 to 23.	1-30
Y	WO,A, 86/01533 (CELLTECH LIMITED), 13 March 1986 (13.03.86), see pages 3-8 and 19-22.	1-30
Y	The Journal of Immunology, Vol.126, No.4, issued April 1981 (USA), UCHIYAMA et al, "A Monoclonal Antibody (ANTI-Tac) Reactive with Activated and Functionally Mature Human T Cells," see pages 1393 to 1397.	1-30

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

10 July 1989

Date of Mailing of this International Search Report

27 JUL 1989

International Searching Authority

ISA/US

Signature of Authorized Officer

Jeff P. Kushan